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ALLELIC VARIABILITY IN THE CYP11B2 C344T SINGLE NUCLEOTIDE
POLYMORPHISM FROM A COHORT OF EAST AFRICANS

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

the Degree Bachelor of Science with

Honors College Graduate Distinction at Western Kentucky University

By

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2014

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ABSTRACT

Non-communicable disease (NCD), in particular cardiovascular disease, is a significant problem in developing countries. Essential hypertension (EH) is a leading risk factor for vascular disease and while managing EH in developing countries is considered a high global priority, few studies exist from third world populations. From a cohort of Kenyans living in the Kasigau region, we have investigated the allele frequency of a single nucleotide polymorphism (SNP) previously reported to correlate with salt-sensitive EH. The SNP being investigated is aldosterone synthase CYP11B2 C344T (rs1799998), a polymorphism in the renin-angiotensin system (RAS). The C344T polymorphism is located in the promoter region of CYP11B2 and affects the production of aldosterone. In this study the overall allele frequency of T=0.81 and C=0.19, and the genotype frequency was T/T=0.66, T/C=0.30, and C/C=0.04 for the total Kasigau population in this study. There was no significant variance in blood pressure among any of the three genotype.

Keywords: Hypertension, Kenya, Cardiovascular Disease, Aldosterone Synthase, Renin-Angiotensin System

Dedicated to my family and the people of Kasigau

“I can do all things through Christ who strengthens me.”

Philippians 4:13

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FIELDS OF STUDY

Major Field: Biology
Concentration: Pre-Dental

Minor Field 1: Chemistry

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TABLE OF CONTENTS

	<u>Page</u>
Abstract	ii
Dedication	iii
Acknowledgements	iv
Vita.....	v
List of Figures	viii
List of Tables	ix
Chapters:	
1. Introduction.....	1
2. Materials and Methods.....	7
3. Results.....	12
4. Discussion.....	17
Appendix A.....	20
Appendix B	21
Appendix C	22
References.....	23

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
Figure 1: Renin-angiotensin system.....	3
Figure 2: Healthcare infrastructure in Kenya.....	4
Figure 3: Kasigau, Kenya Sub-Locations	5
Figure 4: EH Distribution in Kasigau, Kenya.....	6
Figure 5: 2.1 Taqman® genotyping assay	10
Figure 6: CYP11B2 allele frequencies for Kasigau.....	14
Figure 7: CYP11B2 genotype distribution for Kasigau.....	14

LIST OF TABLES

<u>Table</u>	<u>Page</u>
Table 1: CYP11B2 genotype distribution and allele frequencies per EH classification in Kasigau	15
Table 2: Variance in systolic blood pressure among genotype groups.....	16

CHAPTER 1

INTRODUCTION

In 2008 the World Health Organization estimated that over 36 million deaths occurred worldwide from non-communicable diseases (NCD). Out of the 57 millions deaths that occurred worldwide, NCD were the leading cause of mortality at 63% (World Health Organization, 2011). NCDs are diseases that cannot be transmitted from person to person and include, cardiovascular disease (CVD), cancer, chronic respiratory disease, stroke, and diabetes. NCD has been known to be the leading cause of pre-mature death in people under 60 years old. In lower-middle-income countries NCD causes 28% of the deaths of people under 60 years old, and in lower-income countries it causes 41% of the pre-mature deaths under 60. One of the risk factors associated with NCD is essential hypertension (EH) (World Health Organization, 2011).

EH is defined as high blood pressure without a known cause (Messerli & Williams, 2007). EH is a worldwide concern because of its high frequency and link with CVD (Kearney et al., 2005). EH has been found to be the leading risk factor for CVD (Kobubo, 2014). Of the 39 million people that died of a NCD in 2008, 48% of those deaths were linked to CVD (World Health Organization, 2011).

There are varying stages of hypertension classified as normotensive, pre-hypertension, stage 1 hypertension, and stage 2 hypertension. Blood pressure consists of a systolic (resulting from ventricular contraction) measurement and diastolic (ventricular

relaxation) measurement, which is quantified in mmHg. Normal blood pressure is less than or equal to 120/80 mmHg. Pre-hypertensive individuals have a blood pressure between 121/81-139/89 mmHg. Stage 1 hypertension is classified as 140/90-159/99, and stage 2 hypertension is classified as anything greater than 160/100. There is a direct relationship between an increase in blood pressure and a greater risk for CVD.

EH is a complex polygenic disease that has been linked to many environmental and hereditary factors in humans (Doris, 2002). Studies have shown that hypertension has been connected to a collection of single nucleotide polymorphisms (SNPs) (Doris, 2002 & Kumar et al., 2003). A SNP is a common single nucleotide variation in the DNA sequence of species. It has been found that single nucleotide variation can alter a person's susceptibility to hypertension (Doris, 2002).

When a polymorphism or SNP is identified it is referenced with a SNP ID, rs number. An rs number is defined as a variation at a location on an ideal reference chromosome and archived in the Single Nucleotide Polymorphism Database (dbSNP). The database was developed and managed by the National Center for Biotechnology Information of the National Institutes of Health. The specific SNP in this study is the CYP11B2 C344T (rs1799998), which has been linked to EH in humans in other studies (Kumar et al., 2003). CYP11B2 is an aldosterone synthase in the renin-angiotensin system (RAS) (Kumar et al., 2003).

RAS is the key hormonal system in the body responsible for regulating blood pressure and sodium homeostasis (Giner et al., 2000). It has a series of enzymes and genes that are found throughout the body that can lead to elevating blood pressure (Figure 1). Angiotensinogen is secreted from the liver and broken down to angiotensin 1 by

renin, and then converted to angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II is responsible for numerous physiological effects including the secretion of hormone aldosterone from the adrenal cortex. Aldosterone in the RAS has been found to control the reabsorption of ions and water in the kidney. As reabsorption of ions and water increases blood pressure eventually resulting in hypertension. The C344T polymorphism is located in the promotor region of the CYP11B2 gene and affects aldosterone production because the gene encodes aldosterone synthase catalyzes the final step in aldosterone biosynthesis (Kumar et al., 2003).

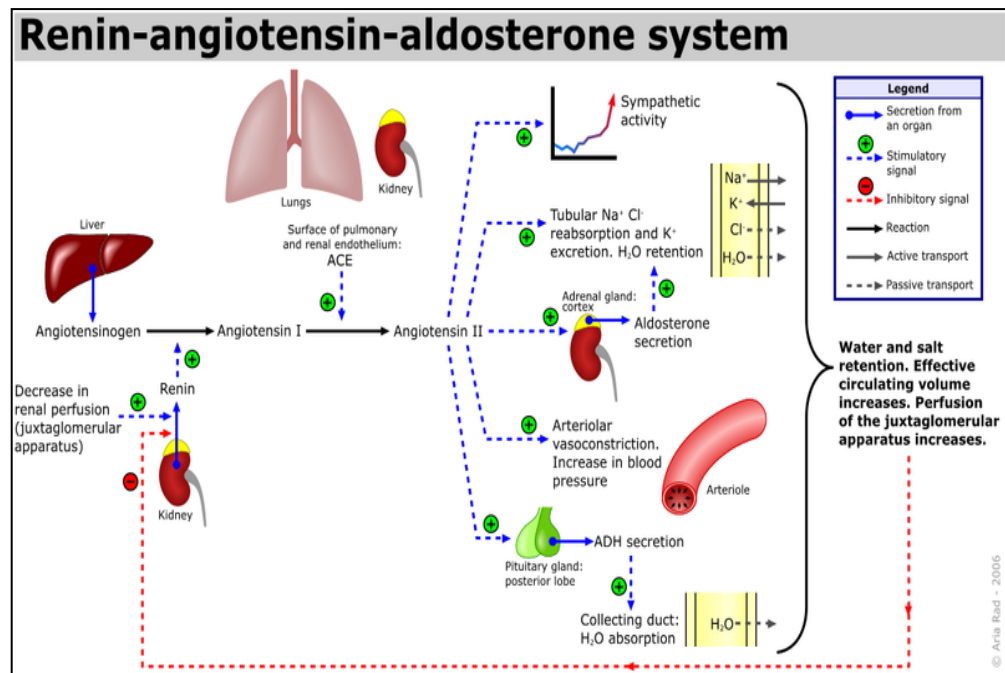


Figure 1: Renin-angiotensin system. The arrows indicate the hormonal pathway that can lead to hypertension through Angiotensin II controlling the release of Aldosterone (Public Domain)

This study is based in Kenya, a sub-Saharan country in east Africa, with a population of approximately 43 million people (World Health Organization, 2012). About 75-80% of Kenyans live in a rural area on less than one US dollar per day (Unicef, 2014). The Kenyan health care system is a pyramid-like structure with only two national

referral hospitals. The rest of the country is divided into districts that have a few district-level hospitals and provincial clinics. Rural health centers and dispensaries provide most of the primary health care (Figure 2). The health care system is government run by the Ministry of Health and the Ministry of Public Health & Sanitation. On average there are 18 doctors per 100,000 people within the country (The World Health Organization, 2011). This is drastically different from the United States with on average 242 doctors per 100,000 people (The World Health Organization, 2011).

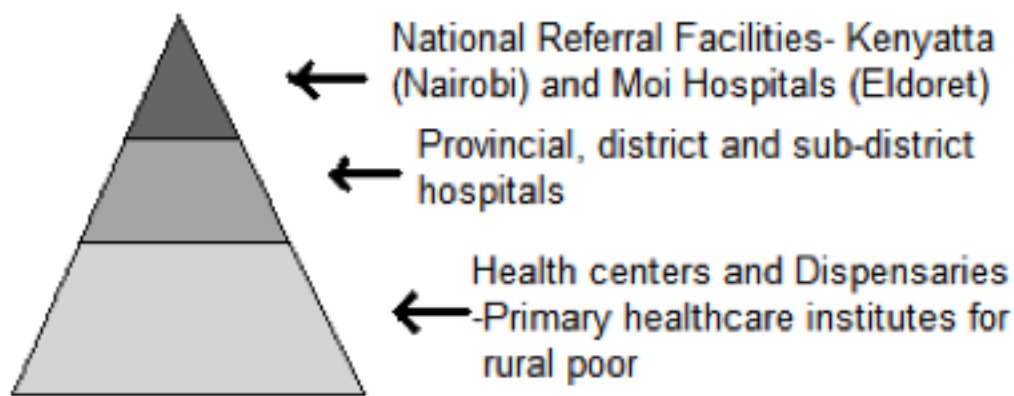


Figure 2: Healthcare infrastructure in Kenya (Williams, 2012)

This study takes place in Kasigau, Kenya. Kasigau is located in the Taita-Taveta District of southern Kenya. Kasigau is further divided into three sub-locations and seven total villages (Figure 3). Sub-location Rukanga contains 29% of the population, and it includes four villages: Rukanga, Jora, Ngambenyi, and Bungule. Sub-location Makwasinyi makes up 21% of the total population spread across three villages: Makwasinyi, Kisimenyi, and Kiteghe. The third sub-location is Buguta, which contains 50% of the Kasigau population (Census data, 2009). It has been found that through previous research in our lab that there is a high prevalence of EH in Kasigau, Kenya

compared to populations in the United States (Figure 4) (Williams, 2012; Wright et al., 2011).

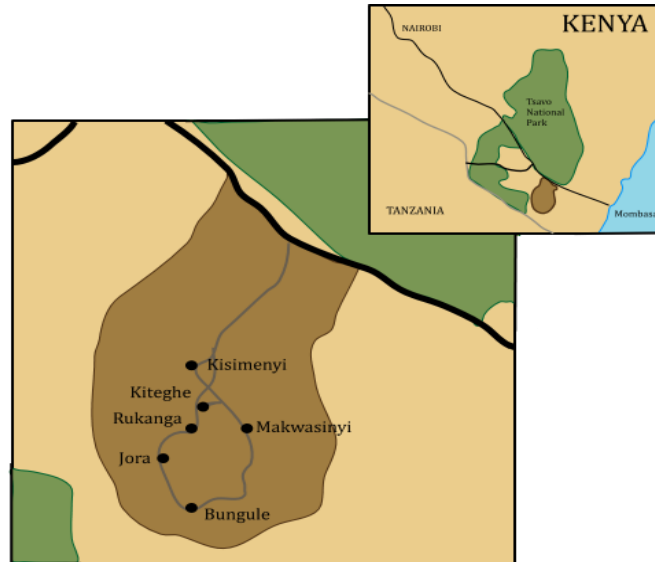


Figure 3: Kasigau, Kenya Sub-Locations (<http://www.kids4kenya.org/TheSchools.php>)

Through Western Kentucky University's Partners in Caring: Medicine in Kenya (PiC:MiK) program the health care concerns of hypertension within the Kasigau area are met. PiC:MiK is a partnership between the people of Kasigau, Kenya and Western Kentucky University. A team of approximately 20 people (3 doctors, 14 students, 1 faculty member, and 2 volunteers) travel to Kenya for two weeks to conduct medical clinics and health care education through sustainable techniques and community oriented work. PiC:MiK also offers the pre-professional students on the trip a first hand global view of health care in a developing country. The 2014 visit was the sixth visit to Kenya by this program in the last seven years. This thesis is a component of the research that is focused on the cardiovascular health of the Kasigau people.

The goal of this research project is to evaluate the prevalence of one SNP (CYP11B2 C344T (rs1799998)) and its correlation to an increased risk of EH in Kasigau, Kenya. It was hypothesized that the high prevalence of observed EH in Kasigau may be linked to the CYP11B2 C344T polymorphism. This study will provide key genetic information that will help prevent, treat, and/or control hypertension in Kenya and other sub-Saharan countries.

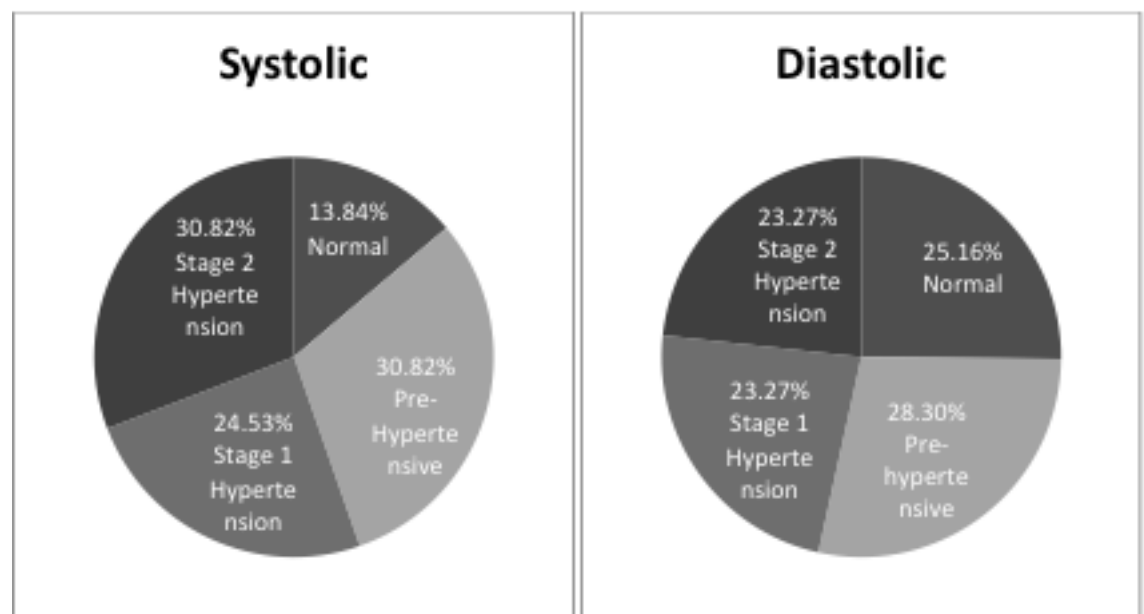


Figure 4: EH Distribution in Kasigau, Kenya: The pie graph on the left shows the values for systolic blood pressure, and the pie graph on the right shows the values for the diastolic blood pressure. (Williams, 2012)

CHAPTER 2

MATERIALS AND METHODS

Compliance/Informed Consent

This study was approved by Western Kentucky University's Human Subjects Review Board (Hr-174, Appendix A) and the Taita District Health Officer in Kenya (Appendix B). All participants were read an informed consent document and their willingness to participate in the study implied their consent (Appendix C).

Study Participants

Participants for this study were selected using a cross-sectional population assessment of the Kasigau, Kenya using census data of the region. The area was subdivided into sublocations: Rukanga, Makwasinyi, and Bughuta which comprise 29%, 21%, and 50% of the total population, respectively. Each sublocation includes one to four villages in the Kasigau area.

Each participant was between the ages of 45-75 because of the increased risk of EH in this age group. The goal was to have 50% of participants to be male and 50% of participants to be nonpregnant females. However, 66% of the participants were female and 34% were males. Overall, 400 individuals were recruited to take part in the study, although only 161 of those individuals participated in the study.

Buccal Cell Acquisition

Buccal cells were acquired from participants using Isohelix DNA Buccal Cell Swabs. These swabs were excellent to collect human genomic DNA because they can yield up to 5 µg of DNA. The swabs were labeled by village and participants number in order to correlate genotype data to an individual's demographic data.

The collection of the buccal cells occurred during the participant's clinic visit. The individual's cheek was swabbed thoroughly for one minute, and then the swabs were sealed to avoid contamination. The samples were stored at 4°C until the DNA was isolated.

Genomic DNA Isolation

Genomic DNA was isolated from swabs by standard alkaline lysis using the QIAamp DNA Mini Kit (Qiagen; Valencia, CA, USA). Briefly, swabs were submerged in phosphate buffered saline (PBS) in a microcentrifuge tube. Proteinase K and Buffer AL were added and the samples were immediately vortexed for fifteen seconds to ensure efficient lysis. Proteinase K was selected as the optimal enzyme with the lysis buffer (Qiagen; Valencia, CA, USA). The samples were incubated at 56°C for ten minutes, which activated the protease within each sample.

Subsequently ethanol (96-100%) was added to each sample, vortexed to ensure proper mixing, then pipetted onto a spin column from the QIAamp DNA Mini Kit, and centrifuged at 6,000 g, 1 minute to isolate genomic DNA. Columns were washed and centrifuged with a series of wash buffers (AW1 and AW2) provided in the kit. The DNA was extracted from the spin columns by the addition of buffer AE, by heating to 60°C.

The columns were left to incubate with buffer AE in the tube for five minutes before they were centrifuged at 6,000 g for one minute. The elution step was performed twice to increase DNA yield. The two sets of DNA from each sample were stored at -20°C. All genomic DNA was isolated from samples prior to quantification.

Nanodrop Analysis

DNA concentration and purity were determined by the ratio of absorbance of UV light at 260/280 nm using a Nanodrop spectrophotometer (Thermo Scientific; Wilmington, DE, USA). Two µl of each sample was analyzed to determine the final DNA concentration.

Allelic Discrimination Analysis

Quantitative Polymerase Chain Reaction (Q-PCR) was used for genotype and allelic discrimination, specifically by using the Taqman SNP Genotyping Assay Protocol (Applied Biosystem; Foster City, CA, USA). This assay uses fluorescent dyes to determine DNA binding. Each dye is covalently attached to a primer used in the PCR reaction. A blue dye, 6FAM indicates the fluorescence of the G allele in the SNP. The green dye, VIC indicates the fluorescence of the A allele in the SNP. Sequence Detection System (SDS) software allowed the identification of the blue or green fluorescence (Applied Biosystems; Foster City, CA, USA). A reporter dye and a quencher dye are also found within the assay (Figure 5).

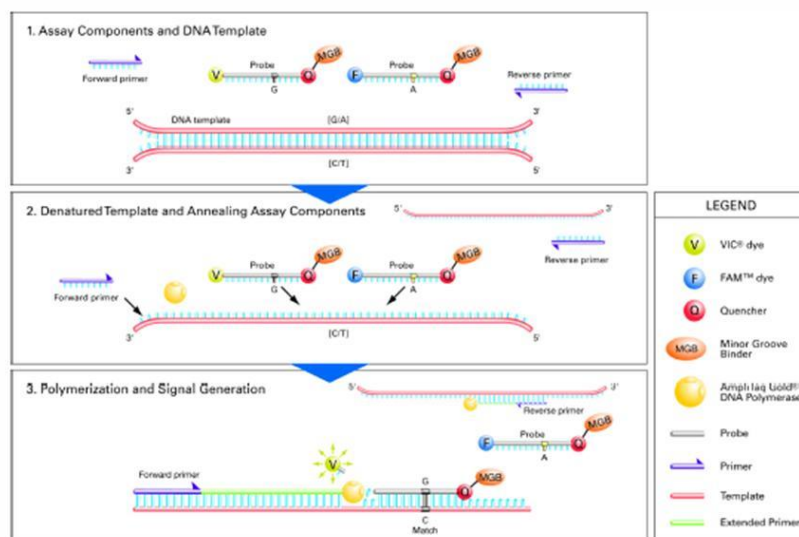


Figure 5: Taqman® genotyping assay. (“Taqman® Genotyping Assay Product Bulletin,” Applied Biosystems; Foster City, CA, USA).

All samples were run in triplicate on a MicroAmp® Optical 96-well reaction plate (Applied Biosystems; Foster City, CA, USA) in an Applied Biosystem Prism 7300 Real-Time polymerase chain reaction instrument. On each plate a negative control was included that lacked DNA.

Each assay included 12.5 µl of a premade master mix, 1.25 µl of SNP assay, 11.25 µl of DNA and water, as well as 50 nM of forward primer labeled with VIC dye, 50 nM of reverse primer labeled with FAM dye, deoxyribonucleotides with deoxyuridine triphosphates, AmpliTaq Gold DNA polymerase, AmpErase® uracil-N-glycosylase, optimized buffer, and passive reference components. The SNP’s context sequence is provided in the table below.

SNP	Context Sequence
CYP11B2 rs1799998	TTTATCTTATCGTGAGATGAGAGGG[A/G]GCCTTGGATTCTTTTAA TAGACTTT

The cycling conditions were as follows: activation (95°C-10 minutes), denaturing (92°C-15 seconds), extending and annealing (60°C-70 seconds). The cycling procedure was repeated 50 times and was performed to sufficiently elevate the fluorescence for SDS to detect within each well.

Fluorescence intensity for each sample was quantified through Q-PCR. Allele T or C was determined based upon the values given from the fluorescence intensity. Values below the no template control were eliminated from analysis.

Statistical Analysis

The Hardy-Weinburg Equilibrium (HWE) equation was used to determine if the genotype and allelic frequencies observed were affected by any evolutionary influences. The expected values of alleles were derived from observed data and analyzed using the HWE equation ($p^2 + 2pq + q^2 = 1$). The p^2 equals the frequency of the genotype T/T, the p equals the frequency of allele T, the q^2 equals the frequency of the genotype C/C, and the q equals the frequency of allele C.

The genotype frequencies analyzed using a Pearson's Chi Square test. The equation $\chi^2 = \sum \frac{(o - e)^2}{e}$ was used to determine significance of deviation in the genotype frequencies and the HWE models. A Chi Square value was calculated in order to determine if a frequency from each sublocation and in each hypertension classification fell within HWE.

Variance of systolic blood pressure for each genotype was assessed by Kruskal-Wallis one way Analysis of Variance on Ranks using Sigma Plot software.

CHAPTER 3

RESULTS

As a whole, approximately 81% of the Kasigau population possess the T allele, while 19% possess the C allele of the CYP11B2 C344T SNP (Figure 6). Per sublocation the C allele ranged from 13%-23% with Buguta having the lowest frequency. When the allele frequencies were assessed as a genotype, it was found that Kasigau displayed 66% T/T, 30% C/T, and 4% T/T (Figure 7). Makwasinyi had the highest distribution of C/C at 10%.

To assess whether allelic frequency varied based on the severity of EH, the derived allelic and genotypic distribution of the SNP was determined for normotensive, pre-hypertensive, Stage I EH, and Stage II EH, for the total Kasigau population as well as the three sublocations. The EH classification was determined by using only the systolic BP of participants. When the obtained genotype frequencies of the C344T SNP were compared to the expected frequencies predicted by the HWE, all alleles were found to be in HWE with the exception of pre-hypertensive people from Makwasinyi, ($\chi^2=8.00$, $p=0.04$).

For all the sublocations and EH classifications T alleles were in greater frequency than C alleles (Table 1). The T allele frequency for Kasigau as a whole, Buguta, Makwasinyi, and Rukanga ranged from 78%-87%, while the C allele frequency ranged from 13%-22%. The majority of the participants in the study had a genotype of T/T.

When variance in systolic blood pressure was analyzed among the three different genotypes there was no significant difference among them, as determined by the ANOVA, ($p=0.78$) (Table 2). The difference in the median values among the treatment groups is not great enough to exclude the possibility that the difference is due to random sampling variability. However, variance was not analyzed based on EH stage.

Based upon the results, the majority of the people of Kasigau possess the T allele of the CYP11B2 C344T SNP. There is small variability in the frequency of the alleles with the Kasigau participants who are normotensive vs the participants who have some form of hypertensive. More statistical analysis will need to be done to confirm these results.

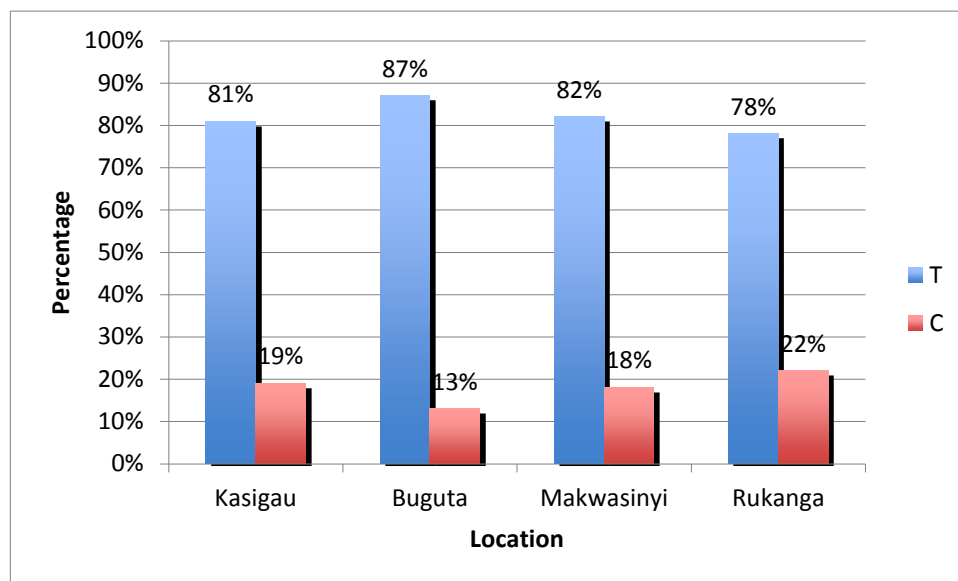


Figure 6: CYP11B2 allele frequencies for Kasigau

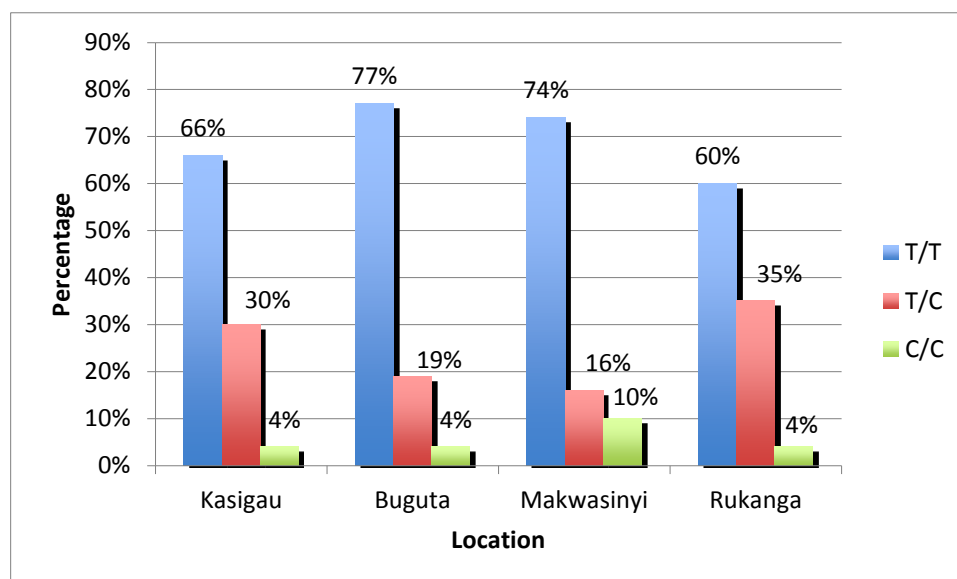


Figure 7: CYP11B2 genotype distribution for Kasigau

Table 1: CYP11B2 genotype distribution and allele frequencies per EH classification in Kasigau. HWE, Hardy-Weinburg Equilibrium. *=p≤0.05

Rukanga	Genotype	Normotensive	Pre-hypertensive	Type 1 EH	Type 2 EH
N=48	T/T	6	10	5	8
	T/C	2	6	5	4
	C/C	1	0	1	0
	HWE	$\chi^2=1.15$ p=0.28	$\chi^2=0.85$ p=0.36	$\chi^2=0.02$ p=0.87	$\chi^2=0.48$ p=0.49
	Allele	Normotensive	Pre-hypertensive	Type 1 EH	Type 2 EH
	T	14	26	15	20
	C	3	6	6	4
	f (T)	0.82	0.81	0.71	0.83
	f (C)	0.18	0.19	0.29	0.17
Makwasinyi	Genotype	Normotensive	Pre-hypertensive	Type 1 EH	Type 2 EH
N=31	T/T	0	7	5	11
	T/C	0	0	3	2
	C/C	0	1	1	1
	HWE	$\chi^2=ND$ p=ND	$\chi^2=8.00$ p=0.04*	$\chi^2=0.26$ p=0.61	$\chi^2=2.43$ p=0.12
	Allele	Normotensive	Pre-hypertensive	Type 1 EH	Type 2 EH
	T	0	14	13	24
	C	0	2	5	4
	f (T)	0.00	0.88	0.72	0.86
	f (C)	0.00	0.13	0.28	0.14
Bughuta	Genotype	Normotensive	Pre-hypertensive	Type 1 EH	Type 2 EH
N=26	T/T	3	9	5	3
	T/C	0	3	1	1
	C/C	0	1	0	0
	HWE	$\chi^2=ND$ p=ND	$\chi^2=0.86$ p=0.35	$\chi^2=0.05$ p=0.82	$\chi^2=0.08$ p=0.77
	Allele	Normotensive	Pre-hypertensive	Type 1 EH	Type 2 EH
	T	6	21	11	7
	C	0	5	1	1
	f (T)	1.00	0.81	0.92	0.88
	f (C)	0.00	0.19	0.08	0.13
Kasigau	Genotype	Normotensive	Pre-hypertensive	Type 1 EH	Type 2 EH
N=105	T/T	9	26	15	22
	T/C	2	9	9	7
	C/C	1	2	2	1
	HWE	$\chi^2=1.92$ p=0.17	$\chi^2=0.95$ p=0.33	$\chi^2=0.15$ p=0.69	$\chi^2=0.22$ p=0.64
	Allele	Normotensive	Pre-hypertensive	Type 1 EH	Type 2 EH
	T	20	61	39	51
	C	4	13	13	9
	f (T)	0.83	0.82	0.75	0.85
	f (C)	0.17	0.18	0.25	0.15

Table 2: Variance in systolic blood pressure among genotype groups; $H = 0.497$ with 2 degrees of freedom. ($P = 0.780$)

Group	N	Median
T/T	73	140.00
T/C	30	144.00
C/C	7	139.00

CHAPTER 4

DISCUSSION

In this study, the allelic and genotype frequencies of the CYP11B2 C344T SNP known to influence predisposition to EH, was evaluated from a population in rural Kasigau, Kenya. The allele frequencies were compared across normotensive, prehypertensive, Stage I hypertensive, and Stage II hypertensive. Hypertension was measured using only the participants' systolic blood pressure. Overall, this work investigates how polymorphic variation in a RAS gene influences phenotypic differences of EH in Kasigau, Kenya.

There are many different approaches that exist for studying genetic associations of complex diseases including genome-wide association studies, linkage disequilibrium, and pooling strategies (Binder, 2006). The target gene approach was used in this study because SNPs in the RAS genes have already been shown in other populations to specifically correlate with an increase in susceptibility to EH (Tabor et al., 2002).

In our study all of the sublocation/EH classifications were found to be in HWE except for the pre-hypertensive people from Makwasinyi, ($\chi^2=8.00$, $p=0.04$). These results likely reflect limiting sample size although non-random mating may also be contributing. This may be due to the physical location of this village on the opposite side of the Kasigau Mountain, which contributes to its geographic isolation.

It has been found in many studies that the CYP11B2 C344T SNP is associated with EH, but there are differences regarding which allele is the susceptible allele (Sookoian et al., 2007; Kumar et al., 2003; Tiago et al., 2003). Kumar's research study found that the C allele is the susceptible allele for EH in a Caucasian population. The study also found that in participants who had both the C allele and EH, greater than 50% were C/C homozygous. This was found to be significant for women who had EH (Kumar et al., 2003). In the current study, the gender of the participants was not taken into account during the analysis, although 56 of the participants had Stage I or Stage II Hypertension. Out of those 56 participants 16 had the genotype T/C and 3 had the C/C genotype. If a larger sample size were available it is possible that a greater amount of the susceptible C allele may be present.

In contrast, a different study found that the T allele was correlated with an increase in EH in people who were of African ethnicity (Tiago et al., 2003). In Tiago's (2003) study, it was also found that the T allele is more present in a South African population. Sixty-six percent of participants in the current study had the T/T genotype. Our study also showed that 88% of participants with the T/T genotype and 83% of participants with the C/C genotype had EH. Taking into account that all the participants in this study are of African ethnicity our data supports the conclusion of Tiago, et al (2003). However, further research should be done to determine the genetic variation in South Africans and East Africans. It should be noted that the ancestral allele is the T allele. Most of the participants in our study with a form of EH had the T allele, however there is no evidence to disregard the C allele as the susceptible allele. If a larger sample

size were available it is possible that a greater amount of the susceptible C allele may be present.

This study is one of the first to research a SNP related to EH in East Africans (Freeman, 2013). The results in the future will be used to help promote awareness and treatment for hereditary factors causing CVD and EH in developing countries. This study also gave insight into how the genetic components of the RAS potentially affect the development of EH in Kasigau, Kenya. Future research will also include epigenetic factors, gene-gender interactions, aging effects, and salt sensitivity, which all have been shown to be linked to EH in other studies (Deng, 2007; Hendriks et al., 2012).

APPENDIX A
Western Kentucky University Institutional Review Board Approval
(HR-174)



A LEADING AMERICAN UNIVERSITY WITH INTERNATIONAL REACH
HUMAN SUBJECTS REVIEW BOARD

In future correspondence, please refer to HS11-174, March 4, 2011

Dr. Rice
Biology
WKU

Dr. Rice:


Your research project, *The Molecular Epidemiology of Essential Hypertension in Kasigau, Kenya*, was reviewed by the IRB and it has been determined that risks to subjects are: (1) minimized and reasonable; and that (2) research procedures are consistent with a sound research design and do not expose the subjects to unnecessary risk. Reviewers determined that: (1) benefits to subjects are considered along with the importance of the topic and that outcomes are reasonable; (2) selection of subjects is equitable; and (3) the purposes of the research and the research setting is amenable to subjects' welfare and producing desired outcomes; that indications of coercion or prejudice are absent, and that participation is clearly voluntary.

1. In addition, the IRB found that you need to orient participants as follows: (1) signed informed consent is not required; (2) Provision is made for collecting, using and storing data in a manner that protects the safety and privacy of the subjects and the confidentiality of the data. (3) Appropriate safeguards are included to protect the rights and welfare of the subjects.

This project is therefore approved at the Expedited Review Level until March 4, 2012.

2. Please note that the institution is not responsible for any actions regarding this protocol before approval. If you expand the project at a later date to use other instruments please re-apply. Copies of your request for human subjects review, your application, and this approval, are maintained in the Office of Sponsored Programs at the above address. Please report any changes to this approved protocol to this office. A Continuing Review protocol will be sent to you in the future to determine the status of the project. Also, please use the stamped approval forms to assure participants of compliance with The Office of Human Research Protections regulations.

Sincerely,


Paul J. Mooney, M.S.T.M.
Compliance Manager
Office of Research
Western Kentucky University

cc: HS file number Rice HS11-174



IRB APPLICATION # 11-174
APPROVED 3/4/11 to 3/4/12
EXEMPT EXPEDITED FULLBOARD
DATE APPROVED 3/4/11

APPENDIX B
Taita District Approval

MINISTRY OF PUBLIC HEALTH AND SANTATION

TELEGRAMS: MEDICAL Wundanyi HEALTH
TELEPHONE: 0148 2195
FAX No. 2195
E-mail mohtaveta@africaonline.co.ke
When replying please quote



**MEDICAL OFFICER OF HEALTH,
TAITA DISTRICT,
P.O. BOX 1216,
WUNDANYI**

REF:

Date: 22/2/2011

NANCY A. RICE
DEPARTMENT OF BIOLOGY
WESTER KENTUCKY UNIVERSITY
1906 COLLEGE HEIGHTS BLVD#11080
BOWLING GREEN, KY 42101-1080

Dear Madam

RE: RESEARCH ON HYPERTENSION IN KASIGHAU

This concerns your earlier request for permission to conduct a study on the epidemiology of hypertension in the villages of Kasighau in TAITA District. Your request is granted and we hope to work closely with you and to share your findings.

Yours faithfully,

Dr. Charles G. N. Njoroge
Taita District
P.O. Box 1216
Wundanyi

Dr. Charles G. N.
District Medical Officer of Health
TAITA DISTRICT

APPENDIX C

Informed Consent Document

INFORMED CONSENT DOCUMENT

Project Title: The Molecular Epidemiology of Essential Hypertension in Kasigau, Kenya
Investigators: Nancy Rice, Biology, 270-745-5995



To Be Read to All Potential Participants:

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your agreement to participate in this project. We will explain the project to you in detail including the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask any questions you have to help you understand the project.

This project is designed to assess the prevalence of high blood pressure and its associated risk factors in the Kasigau area of Kenya. The main objective of this study will be to test the hypothesis that a high prevalence of salt-sensitive hypertension exists in the people of Kasigau, Kenya which results from genetic differences in the hormonal system known to regulate salt excretion called the renin-angiotensin gene system.

To test this hypothesis, we will measure the prevalence and current management of hypertension and evaluate the frequency of common environmental risk behaviors associated with high blood pressure. This will involve taking several noninvasive measurements of blood pressure, height, weight, and pulse rate. We will also collect a small blood sample by finger stick to measure glucose levels- to test for diabetes, and lipid levels- to test for high cholesterol. We will also take one larger blood sample by a needle venous puncture to look for genetic variation in certain genes known to be associated with high blood pressure. If you are found to have high blood pressure, you may also be asked to provide a 24-h urine sample for salt analysis.

Following sample collection, you will be asked a series of questions. Please answer to the best of your ability and as accurately as possible. There will be only minimal discomfort and no risk associated with this project. Ultimately your information, along with that of all participants, will be used to determine key epidemiological and genetic information regarding mechanisms that lead to hypertension in the people of Kasigau, Kenya; this new scientific knowledge will be directly translatable into prevention, treatment, and control of hypertension and will ultimately be used to enhance the health and well-being of the people of Kasigau.

All participants will be assigned a number to maintain anonymity. No names will be recorded or presented in the data. Refusal to participate in this study will have no effect on any future services you may receive from WKU. Anyone who agrees to participate in this study is free to withdraw at any time with no penalty.

Your continued participation in the study will imply your consent. Do you wish to continue?

THE DATED APPROVAL ON THIS CONSENT FORM INDICATES THAT
THIS PROJECT HAS BEEN REVIEWED AND APPROVED BY
THE WESTERN KENTUCKY UNIVERSITY HUMAN SUBJECTS REVIEW BOARD
Paul Mooney, Compliance Coordinator
TELEPHONE: (270) 745-4652

IRB APPLICATION # 11-174
APPROVED 3/4/11 to 3/4/12
EXEMPT EXPEDITED FULLBOARD
DATE APPROVED 3/4/11

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